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Genetic association of bipolar disorder with the β_3 nicotinic receptor subunit gene

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Abstract

Objective—Owing to the clinical relationship between bipolar disorder and nicotine dependence, we investigated two research questions: (i) are genetic associations with nicotine dependence different in individuals with bipolar disorder as compared with individuals without bipolar disorder, and (ii) do loci earlier associated with nicotine dependence have pleiotropic effects on these two diseases.

Method—Our study consisted of 916 cases with bipolar disorder and 1028 controls. On the basis of known associations with nicotine dependence, we genotyped eight single-nucleotide polymorphisms (SNPs) on chromosome 8 (three bins) in the regions of CHRNA3 and CHRNA6, and six SNPs on chromosome 15 (three bins) in the regions of CHRNA5 and CHRNA3.

Results—To determine whether the genetic associations with nicotine dependence are different in bipolar disorder than in the general population, we compared allele frequencies of candidate SNPs between individuals with nicotine dependence only and individuals with both nicotine dependence and bipolar disorder. There were no statistical differences between these frequencies, indicating that genetic association with nicotine dependence is similar in individuals with bipolar disorder as in the general population. In the investigation of pleiotropic effects of these SNPs on bipolar disorder, two highly correlated synonymous SNPs in CHRNA3, rs4952 and rs4953, were significantly associated with bipolar disorder (odds ratio 1.7, 95% confidence interval: 1.2–2.4, P

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= 0.001). This association remained significant both after adjusting for a smoking covariate and analyzing the association in nonsmokers only.

Conclusion—Our results suggest that (i) bipolar disorder does not modify the association between nicotine dependence and nicotinic receptor subunit genes, and (ii) variants in *CHRNA3*/*CHRNA6* are independently associated with bipolar disorder. Psychiatr Genet 00:000–000.

Keywords

analyses; *CHRNA3*; *CHRNA5*; *CHRNA6*; genetic association; nicotine; tobacco use disorder

Introduction

There is significant comorbidity between nicotine dependence and bipolar disorder. Not only are individuals with bipolar disorder prone to smoking (Gonzalez-Pinto *et al.*, 1998), but lifetime smoking in bipolar disorder is associated with earlier age of onset of symptoms, greater severity of symptoms, decreased functioning, history of suicide attempts, and comorbid anxiety and substance use disorders (Ostacher *et al.*, 2006). Additional studies described lower quality of life in bipolar disorder associated with nicotine dependence (Gutierrez-Rojas *et al.*, 2008), and poor treatment outcome in bipolar mania for smokers as compared with nonsmokers (Berk *et al.*, 2008).

Evidence for genetic factors contributing to the risk of smoking behaviors and nicotine dependence is seen in the clustering of heavy smoking and nicotine dependence in families and the similarity of smoking behaviors in identical twins (Bierut *et al.*, 1998; Li, 2006). The most biologically compelling associations with nicotine dependence have been found in the $\alpha 5$ nicotinic receptor subunit gene *CHRNA5* on chromosome 15 (Bierut *et al.*, 2007; Saccone *et al.*, 2007). This region also encompasses the nicotinic receptor subunit genes *CHRNA3* and *CHRNA4* in addition to the genes *IREB2* and *PSMA4*. This association has been replicated in many other independent studies (Berrettini *et al.*, 2008; Spitz *et al.*, 2008; Thorgeirsson *et al.*, 2008; Weiss *et al.*, 2008; Saccone *et al.*, 2009). A second region on chromosome 8 including the nicotinic subunit receptor genes *CHRNA3* and *CHRNA6* has also been associated with nicotine dependence (Bierut *et al.*, 2007; Saccone *et al.*, 2007). This association was recently confirmed in a large metaanalysis (Thorgeirsson *et al.*, 2010).

The molecular and genetic basis for bipolar disorder is just beginning to emerge. Family and twin studies have found strong evidence of heritability (Smoller and Finn, 2003; Kieseppa *et al.*, 2004), but despite significant effort, only two consistent signals were replicated: α -1 subunit of the L-type voltage-gated calcium channel, and Ankyrin 3 (The Wellcome Trust Case Control Consortium, 2007; Baum *et al.*, 2008; Ferreira *et al.*, 2008; Sklar *et al.*, 2008; Smith *et al.*, 2009, in preparation).

In this study, we investigated the relationship between nicotine dependence and bipolar disorder, focusing on the nicotinic receptor subunit genes associated with nicotine dependence: *CHRNA5/CHRNA3/CHRNA4* (chromosome 15q25), and *CHRNA3/CHRNA6* (chromosome 8p11) (Bierut *et al.*, 2007; Saccone *et al.*, 2007, 2008). First, we evaluated whether the association between these genes and nicotine dependence is the same in bipolar disorder as it is in the general population. Then, we investigated the possibility of pleiotropy by evaluating associations between bipolar disorder and variants in *CHRNA5/CHRNA3/CHRNA4* on chromosome 15, and *CHRNA3/CHRNA6* on chromosome 8.

Materials and methods

Participants

Three different study populations were used. A sample of 916 European Americans (EA) with bipolar disorder and 1028 EA controls were recruited for the primary analysis; this is subsequently referred to as the primary data set. The remaining two samples were recruited for replication: 595 EA patients with bipolar disorder and 394 EA controls, and 389 African American (AA) participants with bipolar disorder and 671 AA controls. All bipolar patients were recruited at 11 data collection sites as the fifth wave of data collection by the Bipolar Consortium. Participants were interviewed with the Diagnostic Interview for Genetic Studies (Nurnberger *et al.*, 1994), medical records were obtained, and final bipolar diagnosis was based on the *APA Diagnostic and Statistical Manual*, Fourth edition (American Psychiatric Association, American Psychiatric Association, Task Force on DSM-IV, 2000). Control participants were collected separately through a volunteer panel and web-based psychiatric interview (Sanders *et al.*, 2008). The replication sample (both the EA sample and AA sample) was collected and genotyped as part of the Genetic Association Information Network initiative. These data and corresponding documentation are available at dbGaP (<http://www.ncbi.nlm.nih.gov/dbgap>). The specific population and study characteristics are described in detail elsewhere (Dick *et al.*, 2003; Smith *et al.*, 2009, in preparation).

Smoking covariates were collected in all participants. Nicotine dependence was defined by a commonly used definition of Fagerstrom Test of Nicotine Dependence (FTND) score of four or more (Heatherton *et al.*, 1991), and smoker is defined as having smoked ≥ 100 cigarettes lifetime. Demographics and smoking variables for the primary data set are given in Table 1.

The mean age of participants with bipolar disorder is significantly lower than controls and there are more female participants with bipolar disorder than controls. For this reason, we adjusted for age and sex in our analyses. The participants with bipolar disorder had a higher proportion of smoking (58% of participants with bipolar disorder versus 51% of participants without bipolar disorder) and nicotine dependence (69% of smokers with bipolar disorder are nicotine dependent versus 52% of smokers without bipolar disorder).

Single-nucleotide polymorphism selection

The criteria for selection of candidate genes for the primary analysis were based on earlier studies of the genetics of nicotine dependence and related traits. Eight single-nucleotide polymorphisms (SNPs) were chosen in the regions of *CHRNA3* and *CHRNA6* on chromosome 8 and six SNPs were chosen in the region of *CHRNA5* and *CHRNA3* on chromosome 15 based on the top results from the nicotine dependence genome-wide association study (GWAS) of Bierut *et al.* (2007) and the focused follow-up study of Saccone *et al.* (2007). One hundred and seventy-three SNPs were selected as stratification SNPs. A companion study was designed to evaluate the association of bipolar disorder with several linkage peaks on chromosome 6, as a confirmatory study based on promising results published by other investigators (Schulze *et al.*, 2004). It used 1139 candidate SNPs in an 8Mb region of chromosome 6; results are described elsewhere (Knight *et al.*, 2010). Initial genotyping was carried out by Illumina using GoldenGate Assay and BeadArray technology (www.illumina.com).

Genotyping of the replication sample was performed using the Sequenom MassArray system (Sequenom, San Diego, California, USA). The alleles of the SNPs were discriminated by mass spectrometry. Sixteen candidate SNPs were chosen based on being in perfect linkage disequilibrium (LD) ($r^2=1$) with our original findings (rs4952, and rs4953) in the Hapmap CEU population (Frazer *et al.*, 2007).

Quality control

Both SNPs and participants were evaluated for accuracy of data. Sixty-seven participants were removed from the primary data when genetic data did not match with the reported data: six participants were not phenotypically European, 41 had incomplete diagnoses, and 20 had phenotypic sex that did not match genotypic sex. All SNPs were required to have call rates greater than 99%, have minor allele frequencies greater than 1%, and be consistent with the Hardy–Weinberg equilibrium ($P > 0.001$).

Population stratification analysis

To avoid the detection of artificial associations, we used EIGENSTRAT software (Price *et al.*, 2006) in the primary data to identify subpopulations that are genetically similar. Genotype data for 173 high-performance SNPs were analyzed across all participants. The initial results yielded evidence for three genetic clusters of individuals. As one of the principal components used to construct the clusters was statistically different between participants with bipolar disorder and controls [$P = 0.005$ from a Wilcoxon signed-rank test, computed using SAS software (SAS, 2004)], the association analyses used this principal component as a covariate. To further identify population admixture, the population was anchored with the Hapmap samples of EAs, Yorubans, and Asians. Three individuals (all participants with bipolar disorder) were identified as outliers from the group of EAs. They were therefore dropped from the analysis.

Linkage disequilibrium

We estimated D' and r^2 correlation for all pairs of the 14 SNPs on the same chromosome using an EM algorithm as implemented in the computer program Haploview (version 4.0 <http://www.broad.mit.edu/mpg/haploview/>) (Barrett *et al.*, 2005). The D_0 between any two of the six or eight SNPs on the same chromosome is greater than 0.92. The r^2 values are more variable, ranging from 0.15 to 1.0.

Using the program Tagger (<http://www.broad.mit.edu/mpg/tagger/>) (De Bakker *et al.*, 2005), SNPs were grouped into LD bins; every bin contains at least one tag SNP where the minimum r^2 between each member of the bin is 0.8. Using an r^2 threshold of 0.8, six tag SNPs captured most of the variance of the 14 SNPs. The group of association signals from each bin can then be viewed as a single signal.

Correcting for multiple testing

Although we examined a total of 14 SNPs in this study, the SNPs sharing a chromosome (both the eight SNPs on chromosome 8 and the six SNPs on chromosome 15) were highly correlated. Specifically, there were six LD bins formed by the 14 SNPs. The six bins (three on chromosome 8 and three on chromosome 15) were further correlated with the bins on the same chromosome. As the positive correlation results in fewer than six independent statistical tests, we conservatively set the α cut-off at 0.01 for our initial analysis. Statistical significance for the replication sample was set at $\alpha = 0.05$.

Genetic association analysis

To evaluate whether genetic associations for nicotine dependence are different in bipolar disorder as compared with the general population, a χ^2 test was used to compare allele frequencies between participants with nicotine dependence and bipolar disorder and participants with nicotine dependence, but no bipolar disorder. To confirm genetic association between the SNPs and nicotine dependence within bipolar disorder, we evaluated the subset of the primary data with the diagnosis of bipolar disorder and adequate smoking exposure (lifetime cigarettes ≥ 100). Nicotine dependence was the dependent

variable. A logistic regression model was fit correcting for sex, age, and a population stratification principal component. The SNPs were coded 0, 1, and 2, indicating the number of minor alleles.

To evaluate the association between SNPs and bipolar disorder in the primary data, we used a logistic regression model correcting for sex, age, and a population stratification principal component. The SNPs were coded 0, 1, and 2, indicating the number of minor alleles. As we were looking exclusively at genes that had shown association with nicotine dependence, the interaction with nicotine dependence was of interest. For this reason, we ran three separate analyses. First, the analysis was run with the baseline model (bipolar disorder association with SNP adjusted for age, sex and population stratification), then with a three-level covariate for nicotine dependence (0, never smoker; 1, smoker with FTND <4; 2, FTND ≥ 4), and finally the baseline model using the subsample of nonsmokers only. Logistic regression parameters [including odds ratios (OR) and confidence intervals (CI)] were estimated using PLINK (version 1.05 <http://pngu.mgh.harvard.edu/purcell/plink/>) (Purcell *et al.*, 2007).

Imputation and confirmatory analyses

The goal of the confirmatory analysis was to evaluate the association between bipolar disorder and rs4952/rs4953 in an independent population. The replication sample was originally genotyped using the Affymetrix 6.0 chip (Affymetrix, Santa Clara, California, USA), which does not include rs4952 or rs4953. Using the program SNAP (Johnson *et al.*, 2008) (<http://www.broad.mit.edu/mpg/snap/index.php>), we found 16 SNPs in the genome that had an r^2 of 1 with rs4952 and rs4953. One of these SNPs, rs10104038, is on the Affymetrix 6.0 platform, but it did not pass quality control and hence was not present in either the EA or the AA replication data sets.

The program IMPUTE (Marchini *et al.*, 2007) was used to impute rs4952, rs4953, and the 16 related SNPs (r^2 of 1 with rs4952 and rs4953) in both the replication data sets. EAs were imputed separately from AAs. Initially, we evaluated the imputation in EA based on the original data set. Owing to low minor allele frequency (MAF) (4%), the quality of the imputation was poor as measured by the imputation quality score (Lin *et al.*, 2008, in preparation), the computed imputation accuracy adjusted for chance. However, imputation was carried out in AAs in the SNPs with higher allele frequency. As the association was replicated in the AA population in these imputed SNPs, we genotyped the SNPs in the remainder of the sample and tested both the imputation and association. Power calculations were performed by using Quanto (<http://hydra.usc.edu/gxe/>), assuming a 1 : 1 case-control design and a log-additive model.

Results

To determine whether the association with nicotine dependence was the same in bipolar disorder as in general populations, we ran two analyses. First, we statistically compared allele frequencies in participants with nicotine dependence and bipolar disorder to allele frequencies in participants with nicotine dependence, but no bipolar disorder, and there were no statistically significant differences (Table 2). Second, we evaluated the association between nicotine dependence and the individual SNPs in a subset of the $n=570$ participants with both bipolar disorder and adequate exposure to nicotine (smoked >100 cigarettes lifetime). Owing to the lack of power, no statistically significant associations were found. However, the association with rs16969968, a nonsynonymous SNP in *CHRNA5*, had an estimated OR of 1.3 ($P=0.09$) for the additive model and an estimated OR of 1.54 ($P=0.02$) for the recessive model; ORs similar to published results (Saccone *et al.*, 2007,2008). In addition, the estimated ORs of the remaining SNPs were in the same direction as published

results (Saccone *et al.*, 2008). As these SNPs were chosen for their association with nicotine dependence, the combination of consistent associations with nicotine dependence and lack of differences in allele frequencies for nicotine dependence participants with and without bipolar disorder indicate the risk for nicotine dependence imparted by this genetic region is the same in bipolar disorder as it is in the general population.

To evaluate whether the candidate SNPs are associated with bipolar disorder, three separate analyses were performed (Table 3). In the first analysis, individual SNPs were tested for association with bipolar disorder using the covariates age, sex, and a population stratification factor. An association was seen between bipolar disorder and rs4952 and rs4953. To verify that the result was not as a result of confounding by smoking status, a second analysis added a three-level smoking covariate, which retained statistical significance and did not appreciably change the OR for rs4952 and rs4953. As there may be a component of nicotine dependence or cigarette smoking not accounted for by this three-level smoking covariate, and thus driving the observed association with rs4952 and rs4953, we stratified the data by smoking status and looked only at the nonsmokers. Interestingly, the effects of rs4952 and rs4953 remained strong and statistically significant both by adding the smoking covariate and by evaluating only the nonsmokers. The MAF of both rs4952 and rs4953 in the combined sample of cases and controls is 5.5%.

To validate these results, in the two confirmatory data sets, we imputed rs4952, rs4953, and the 16 SNPs that are in full LD ($r^2=1$) with these SNPs (based on the Hapmap CEU population). The overlapping controls were used to evaluate the imputation results in EAs. Owing to the low allele frequency in the population (3% in the $n=636$ controls with known genotypes), the imputation quality score (Lin *et al.*, 2008, in preparation), a measure of imputation quality adjusted for allele frequency, was tested and indicated poor quality of imputation of that region in EAs. Owing to varying allele frequencies in AAs for these 16 SNPs, the imputation in AAs seemed to be successful for the nine SNPs with minor allele frequencies greater than 5%. Four SNPs (rs13419479, rs10104038, rs16891521, and rs16891530) forming one LD bin in AAs were significantly associated with bipolar disorder ($P=0.007$, OR=1.8). The MAF for these SNPs in AAs is 6% overall, 5% in controls, and 9% in cases. The remaining five SNPs, forming a second LD bin, were not significantly associated with bipolar disorder ($P=0.15$, OR=1.3, MAF overall 8%, MAF bipolar 9%, and MAF controls 7%).

To validate the imputation, we genotyped rs4952, rs4953 and all 16 SNPs in both replication samples (EA and AA). This group of SNPs defines one LD bin in EA, with a MAF of 4%, and three LD bins in AA, where one bin has a MAF of 8% (the bin tagged by rs4952/rs4953 has a 0% MAF in AA). The OR between bipolar disorder and the bin tagged by rs10104038 in AA was similar to our original sample at 1.3 (CI: 1.1–2.1, $P=0.03$). However, the OR between bipolar disorder and this bin in EA was 0.9 (CI: 0.76–2.0, $P=0.46$) likely because of low power from the combination of small sample size and low MAF. The sample size necessary to detect an OR of 1.3 with a population MAF of 4% is 5700 participants (equal number of cases and controls).

Discussion

Despite strong evidence of the heritability of bipolar disorder (Smoller and Finn, 2003; Kieseppa *et al.*, 2004), GWASs have yielded limited results (The Wellcome Trust Case Control Consortium, 2007; Baum *et al.*, 2008; Ferreira *et al.*, 2008; Sklar *et al.*, 2008). As participants with bipolar disorder are much more likely to smoke than controls (Gonzalez-Pinto *et al.*, 1998) and robust genetic associations between nicotine dependence and nicotinic receptor subunit genes have been found, we evaluated whether the genetic

mechanism for nicotine dependence is similar for participants with bipolar disorder as in the controls, and whether nicotinic receptor subunit genes are associated with bipolar disorder.

Our first aim was to investigate the genetic associations of nicotine dependence in participants with bipolar disorder as compared with the general population. Our hypothesis was that the strong genetic associations in the general population would be replicated in a sample of participants with bipolar disorder. Studies have shown a strong association of rs16969968 with nicotine dependence (Bierut *et al.*, 2007, 2008; Saccone *et al.*, 2007, 2008), with evidence for a recessive model (Saccone *et al.*, 2007, 2008). In addition, a functional study showed decreased response to a nicotine agonist in the risk allele of rs16969968 (Bierut *et al.*, 2008). Despite the small sample size, we replicated the recessive finding in rs16969968, and found consistent directions of the ORs for the other SNPs. In addition, participants with nicotine dependence had statistically similar allele frequencies both with and without bipolar disorder. This leads us to believe that the functional association between nicotine dependence and the α_5 , α_3 , α_6 , and β_3 nicotinic receptor subunits is similar for participants with bipolar disorder as it is in the general population.

Our second aim was to evaluate the association of nicotinic receptor subunit genes with bipolar disorder. We were interested in understanding whether there are associations between bipolar disorder and the candidate SNPs and if those associations are confounded by smoking or nicotine dependence. The strongest association we found was with the bin tagged by rs4953, a synonymous SNP in the coding region of the β_3 nicotinic receptor subunit gene (Fig. 1). Although these SNPs have been significantly associated with nicotine dependence in the past (Saccone *et al.*, 2007), the association with bipolar disorder seems to be independent of nicotine dependence. The association remains statistically significant both after controlling for nicotine dependence using a smoking covariate, and by performing the association test on a subset of the data containing only nonsmokers. This association was replicated with rs10104038, a SNP in perfect LD with rs4953 in EA located approximately 20 kb upstream of *CHRNA3*, using a replication sample of AA participants (the second replication sample of EA participants lacked the power to confirm the findings). This indicates that there is likely to be an association of the β_3 nicotinic receptor subunit gene with bipolar disorder independent of smoking or nicotine dependence, and independent of any environmental contribution of smoking to bipolar disorder. The causal variant is unclear because of the size of the LD bin in both EA and AA, but these data suggest that there is a causal variant in LD with these SNPs.

As both nicotine dependence and bipolar disorder are typically diagnosed in young adulthood, and some of the participants were as young as 17 years upon data collection, the younger participants may later develop these disorders, and are thus 'incorrectly classified' as controls at this time. This bias, however, would reduce power by contaminating the 'control' population. Therefore, despite this potential bias in the data, the conclusions are likely valid.

One study has specifically evaluated the association between bipolar disorder and nicotinic receptors. Shi *et al.* (2007) tested the association between *CHRNA3* and bipolar disorder, and did not find a positive association. A careful inspection of their study reveals that the study did not test any SNPs correlated with our findings, and, subsequently would not be expected to find these associations.

Several bipolar disorder GWAS and meta-analyses have been conducted (The Wellcome Trust Case Control Consortium, 2007; Ferreira *et al.*, 2008; Sklar *et al.*, 2008; Scott *et al.*, 2009; Smith *et al.*, 2009, in preparation). One study was genotyped on the Affymetrix 6.0 chip, which includes a single SNP in LD with rs4953, but it did not pass quality control

(Smith *et al.*, 2009, in preparation). Unfortunately, the remainder of the studies were genotyped on the Affymetrix 500K chip or the Illumina HumanHap550 Beadchip, neither of which include SNPs in LD with rs4953 (The Wellcome Trust Case Control Consortium, 2007; Ferreira *et al.*, 2008; Sklar *et al.*, 2008; Scott *et al.*, 2009). Furthermore, we showed in this study that imputation in EAs is inadequate in this region because of low allele frequency and unavailability of correlated SNPs in the region. Therefore, the fact that this region has not been highlighted in earlier studies does not discount our present findings.

The β_3 nicotinic subunit receptor has particular functional relevance from the perspective of bipolar disorder. β_3 is expressed in almost all dopamine neurons and is coassembled with α_6 in dopamine striatal terminals (Gotti *et al.*, 2006). The dopaminergic neurons are often targets of treatment of bipolar mania with antipsychotics. In addition, experiments using knockout mice indicate that loss of the β_3 subunit indirectly causes defects in nicotinic acetylcholine receptor assembly, degradation, and/or trafficking (Gotti *et al.*, 2005). The biological relationship between the β_3 nicotinic subunit receptor and bipolar disorder adds to the strength of these findings.

The association of a nicotinic receptor subunit gene with bipolar disorder has marked clinical implications. It suggests that there may be a joint susceptibility to both nicotine dependence and bipolar disorder, explaining the rate of concordance of the two disorders. As schizophrenia has also increased rates of nicotine dependence (Lasser *et al.*, 2000), is highly heritable (Sullivan *et al.*, 2003), and is more common in families with bipolar disorder (Cardno *et al.*, 2002), nicotinic receptors may play a role in susceptibility to other mental illnesses including schizophrenia. Although we have access to the Genetic Association Information Network schizophrenia dataset (Suarez *et al.*, 2006), we were unable to directly test the hypothesis that this variant contributes to schizophrenia because these SNPs were not genotyped in that dataset (and imputation of these SNPs has been unreliable, as showed earlier).

The cholinergic system is also a target for treatments in attention-deficit hyperactivity disorder, depression, and dementia. Therefore, the association of nicotinic receptor subunit genes with bipolar disorder may not only have implications for diagnosis and treatment of bipolar disorder, but may also impact our understanding of the interrelationships between many psychiatric disorders.

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Fig. 1.

Plot of r^2 values with rs13439479 (y-axis), with respect to chromosome 8 position (x-axis) of 10 single-nucleotide polymorphisms (SNPs) based on (i) the HapMap CEU population (European ancestry) and (ii) the HapMap YRI population (African ancestry). The tag SNPs associated with bipolar disorder (rs4953 and rs4952 in European Americans and rs13439479 in African Americans) are labeled. The 10 SNPs form a single bin in the European population, but two bins in the African population.

Table 1

Demographic characteristics of the primary data set

	Bipolar				Controls			
	All	Nonsmokers	Smokers without nicotine dependence	Nicotine dependent smokers	All	Nonsmokers	Smokers without nicotine dependence	Nicotine dependent smokers
Sample size ^a (% bipolar/controls)	916	384 (41)	165 (18)	367 (40)	1028	508 (49)	251 (24)	269 (26)
Male [*]	359	149	68	142	599	267	150	182
Mean age, years	43.5 (13.2)	42.7 (13.5)	43.9 (13.4)	44.4 (12.7)	52.9 (17.7)	48.7 (17.4)	57.6 (18.1)	56.7 (15.9)
(SD) ^{**}								

Nicotine dependence is defined as FTND ≥ 4 . Smoking is defined as having smoked > 100 cigarettes lifetime.

FTND, Fagerstrom Test of Nicotine Dependence; SD, standard deviation.

t_1 In bipolar participants, as compared with controls, nicotine dependence is more common ($P < 0.001$, χ^2 test), and smokers are more likely to be nicotine dependent ($P < 0.001$, χ^2 test).

* $P < 0.001$ difference in proportion of males between participants with bipolar disorder and controls (χ^2 test).

 $P < 0.001$ for difference in age between participants with bipolar disorder and controls (logistic regression, adjusted for smoking status).

Table 2
Comparison of allele frequencies in participants with nicotine dependence with and without bipolar disorder

rs number	Position	Chromosome	Bin	Gene	Function	Minor allele frequency		P value
						ND without BP (%)	ND and BP (%)	
rs1530847 ^a	42667396	8	a			15.4	15.9	0.77
rs1955185 ^a	42668804	8	b			21.7	24.7	0.28
rs13277254	42669139	8	b			21.7	24.7	0.28
rs13277524	42669214	8	b			21.7	24.7	0.28
rs6474412	42669655	8	b			23.4	26.3	0.28
rs6474413	42670221	8	b	CHRNA3	5' UTR	21.9	24.7	0.67
rs4952	42706222	8	c	CHRNA3	Coding (syn)	3.7	5.9	0.20
rs4953 ^a	42706816	8	c	CHRNA3	Coding (syn)	3.7	5.9	0.20
rs684513	76645455	15	d	CHRNA5	Intron	22.1	20.0	0.36
rs637137 ^a	76661031	15	d	CHRNA5	Intron	24.3	21.4	0.19
rs3743078	76681814	15	d	CHRNA3	Intron	24.7	22.1	0.13
rs16969968 ^a	76669980	15	e	CHRNA5	Missense	35.3	34.7	0.97
rs1051730	76681394	15	e	CHRNA3	Coding (syn)	35.5	34.9	0.99
rs578776 ^a	76675455	15	f	CHRNA3	3' UTR	29.6	27.5	0.41

P value is based on r^2 test of difference between allele frequencies of the two groups.
BP, bipolar disorder; ND, nicotine dependence; syn, synonymous; UTR, untranslated region.
^aTag single-nucleotide polymorphisms.

Table 3
Association analyses between bipolar disorder and candidate single-nucleotide polymorphisms

rs number	Position	Chromosome	Bin	Gene	Function	Minor allele frequency				Smoking as a covariate			Nonsmokers only (n = 892)		
						All (%)	Cases (%)	Controls (%)	P value	OR	95% CI	P value	OR	P value	OR
rs1530847 ^a	42667396	8	a			16.9	17.2	16.6	0.98	1.00	0.8–1.2	0.76	1.03	0.68	0.95
rs1955185 ^a	42668804	8	b			23.9	25.6	22.4	0.09	1.15	1.0–1.3	0.06	1.17	0.38	1.10
rs13277254	42669139	8	b			23.8	25.5	22.3	0.09	1.15	1.0–1.3	0.06	1.16	0.40	1.10
rs13277524	42669214	8	b			23.9	25.6	22.4	0.09	1.15	1.0–1.3	0.06	1.17	0.38	1.10
rs6474412	42669655	8	b			25.3	26.9	23.9	0.11	1.14	1.0–1.3	0.08	1.15	0.30	1.12
rs6474413	42670221	8	b	CHRNA3	5' UTR	23.9	25.6	22.4	0.09	1.15	1.0–1.3	0.06	1.16	0.38	1.10
rs4952	42706222	8	c	CHRNA3	Coding (syn)	4.6	5.8	3.5	0.0011	1.72	1.2–2.4	0.0015	1.71	0.0025	2.08
rs4953 ^a	42706816	8	c	CHRNA3	Coding (syn)	4.6	5.9	3.5	0.0008	1.74	1.3–2.4	0.0011	1.74	0.0017	2.13
rs684513	76645455	15	d	CHRNA5	Intron	21.1	20.6	21.6	0.19	0.90	0.8–1.1	0.22	0.90	0.43	0.91
rs637137 ^a	76661031	15	d	CHRNA5	Intron	22.8	22.1	23.4	0.11	0.88	0.7–1.0	0.13	0.88	0.43	0.91
rs3743078	76681814	15	d	CHRNA3	Intron	23.2	22.9	23.5	0.37	0.94	0.8–1.1	0.30	0.92	0.74	0.96
rs16969968 ^a	76669980	15	e	CHRNA5	Missense	34.0	33.2	34.8	0.36	0.93	0.8–1.1	0.36	0.93	0.21	0.87
rs1051730	76681394	15	e	CHRNA3	Coding (syn)	34.1	33.2	34.8	0.27	0.92	0.8–1.1	0.37	0.93	0.21	0.87
rs578776 ^a	76675455	15	f	CHRNA3	3' UTR	27.8	27.5	28.2	0.30	0.92	0.8–1.1	0.28	0.92	0.37	0.91

The first analysis used the entire sample (n= 2019) and adjusted for age, sex, and a population stratification factor. The second analysis used the same sample but added a three-level smoking covariate (0, never smoked; 1, nonnicotine-dependent smoker; 2, nicotine dependent smoker). The third analysis looked only at the nonsmokers in the sample.

CI, confidence interval; OR, odds ratio; syn, synonymous; UTR, untranslated region.

^aTag single-nucleotide polymorphisms.